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Variation in $\delta^{15}N$ and $\delta^{13}C$ values for forages of Arctic caribou: effects of location, phenology and simulated digestion

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RATIONALE: The use of stable isotopes for dietary estimates of wildlife assumes that there are consistent differences in isotopic ratios among diet items, and that the differences in these ratios between the diet item and the animal tissues (i.e., fractionation) are predictable. However, variation in isotopic ratios and fractionation of $\delta^{13}C$ and $\delta^{15}N$ values among locations, seasons, and forages are poorly described for arctic herbivores especially migratory species such as caribou (Rangifer tarandus).

METHODS: We measured the $\delta^{13}C$ and $\delta^{15}N$ values of seven species of forage growing along a 200-km transect through the range of the Central Arctic caribou herd on the North Slope of Alaska over 2 years. We compared forages available at the beginning (May; $n = 175$) and the end ($n = 157$) of the growing season (September). Purified enzymes were used to measure N digestibility and to assess isotopic fractionation in response to nutrient digestibility during simulated digestion.

RESULTS: Values for $\delta^{13}C$ declined by 1.38 ‰ with increasing latitude across the transect, and increased by 0.44 ‰ from the beginning to the end of the season. The range of values for $\delta^{15}N$ was greater than that for $\delta^{13}C$ (13.29 vs 5.60 ‰). Differences in values for $\delta^{13}C$ between graminoids (Eriophorum and Carex spp.) and shrubs (Betula and Salix spp.) were small but $\delta^{15}N$ values distinguished graminoids (1.87 ± 1.02 ‰) from shrubs (−2.87 ± 2.93 ‰) consistently across season and latitude. However, undigested residues of forages were enriched in $\delta^{15}N$ when the digestibility of N was less than 0.67.

CONCLUSIONS: Although $\delta^{15}N$ values can distinguish plant groups in the diet of arctic herbivores, variation in the digestibility of dietary items may need to be considered in applying fractionation values for $^{15}N$ to caribou and other herbivores that select highly digestible items (e.g. forbs) as well as heavily defended plants (e.g. woody browse). Published in 2017. This article is a U.S. Government work and is in the public domain in the USA.
elements would be more useful for estimating the diet of caribou. The stable isotope ratios of C and N in forage plants were measured before and after simulated digestion to determine whether the indigestible residue sampled from the feces or the assimilated fraction sampled from the tissues were biased in their isotopic ratios. We used total phenols as an index of plant secondary metabolites to examine the effect of these anti-nutrients on any fractionation during simulated digestion.

EXPERIMENTAL

Study area and sampling design

This study was conducted in the summer range of the Central Arctic caribou herd on the North Slope of Alaska from 2011 to 2012 (Fig. 1). We sampled nine sites spread evenly along the Dalton Highway from the Kuparuk River to Prudhoe Bay (Fig. 1). Sites were classified into three ecoregions: Brooks Range, Arctic Foothills, and Coastal Plain. Samples of six preferred forage species (Carex aquatilis, C. bigelowii, Eriophorum vaginatum, Pedicularis spp., Salix pulchra, and S. richardsonii) were collected, when present, every 2 weeks from late May to late September. In addition, we collected samples of Betula nana in 2012, because, although this species does not make up a large part of North Slope caribou diets at present, it may be eaten by caribou more frequently in the future because it is increasing in abundance throughout the Arctic. Indeed, another shrub birch, B. glandulosa, makes up a significant part of caribou diets in Quebec. Forage plants were sampled to mimic caribou browsing and grazing – i.e., for woody browse, easily accessible leaves and twigs were stripped off, while forbs and graminoids were clipped at ground level.

Forage samples were transferred to paper bags and air-dried at ambient temperature (0–22°C) in the field, then air-dried to constant mass in a forced-air oven at 50–55°C when the samples were returned to the laboratory, within 2–6 days of collection. A small subsample (approximately 70 g) of woody browse was immediately frozen in the field.

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Figure 1. Location of study sites within the range of the Central Arctic caribou herd. Sites were located in three ecoregions (Coastal Plain, Arctic Foothills, and Brooks Range) along the Dalton Highway. The distribution of the Arctic ecoregions in Alaska is noted in the inset. [Color figure can be viewed at wileyonlinelibrary.com]
and freeze-dried upon return to the laboratory (model 7755044; Labconco, Kansas City, MO, USA) to test for the presence of plant secondary metabolites (PSM). Dried samples were ground through a #20 mesh (1.27 mm) in a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) or a centrifugal mill (ZM 200; Retsch, Haan, Germany).

**Laboratory analyses**

We measured the total N content of 771 forage samples with an elemental analyzer (CNS2000; LECO, St. Joseph, MI, USA) to select two subsets of samples for the start and end of the phenological curve for each forage at each site and year.[21] The early-season subset (n = 175) were samples with the highest N content whereas the last sample collected at each location comprised the late-season subset (n = 157). Samples from the early- and late-season subsets were analyzed for digestibility of C and N (g digested/g whole) by analyzing the nutrient content of undigested residues after in vitro digestion with purified enzymes by a method that had previously been validated for caribou.[22]

The δ15N and δ13C values of forage samples (%) from the early- and late-season subsets in 2011 (all species) and in 2012 (woody browse species only) were determined with a Europa Scientific 20-20 continuous flow isotope ratio mass spectrometer (Sercon Ltd, Crewe, UK) at the Alaska Stable Isotope Facility, University of Alaska, Fairbanks (Fairbanks, AK, USA). In addition, residues from the in vitro digestibility method were analyzed for their δ15N and δ13C values after extraction with hot water.[11] We used peptone (P7750; Sigma-Aldrich, Milwaukee, WI, USA) as a reference standard to monitor machine drift every 10 assays within each analytical run. Fractionation between the diet-indigestible fraction (Fi) and the δ15N and δ13C values was calculated as:

\[
F_{\text{diet-indigestible}} = \delta X_{\text{residue}} - \delta X_{\text{whole plant}} \tag{1}
\]

Fractionation of the diet-digested fraction for δ15N and δ13C values was calculated by converting each isotope measurement into mass ratios (g isotope/g element) and then multiplying this value by the N and C content (g element/g dry matter), respectively, of whole plants and indigestible residues to obtain the content of 15N, 14N, 13C, and 12C on a dry matter basis.[23] Differences in the content of each isotope between indigestible residues and whole plants were used to calculate the mass ratios and δ15N values, those values were not significantly different among ecoregions (Kruskal-Wallis test; P < 0.01; Table 1; Fig. 3). Values of δ15N were also not significantly different between early and late season although the total N concentration was significantly greater in early- than late-season forages (F1,1290 = 608.11, P > 0.001): graminoids (C. aquatilis, C. bigelovii, and E. vaginatum), woody browse (B. nana, S. pulchra, and S. richardsonii), and forb (Pedicularis sp.). Groups of data are summarized as mean ± standard error (SE) where indicated. We used P < 0.05 as the criterion for significance of α in all comparisons. Bonferroni corrections were applied to determine the significance of multiple post-hoc comparisons.

**RESULTS**

Reference assays for N were not significantly different among analytical runs on the mass spectrometer (n = 5; F4,30 = 0.16) and were not significantly different from the expected δ15N value of 7.00‰ (n = 35; 7.03 ± 0.28; t34 = 0.72). Similarly, reference assays for C were not significantly different among mass spectrometer runs (n = 5; F4,30 = 0.68) and were not significantly different from the expected δ13C value of -15.80‰ (n = 35; -15.77 ± 0.23; t34 = 0.78).

**Spatiotemporal variation in δ15N and δ13C values**

An ANOVA model that included plant group and ecoregion without season provided the greatest inference for explaining variation in δ15N values of forages. Plant groups differed in δ15N values (range = -9.47 to +3.82 ‰; F2,40 = 140.56; P < 0.01; Table 1; Fig. 2), because graminoids were enriched in 15N (1.87 ± 1.02 ‰), whereas woody browse (4.31 ± 2.06 ‰) and Pedicularis spp. (2.87 ± 2.93 ‰) were depleted in 15N. Values of δ15N also varied among species of woody browse (Kruskal-Wallis test; P < 0.01); B. nana was most depleted in 15N (7.38 ± 1.60 ‰), S. richardsonii was intermediate in 15N (4.54 ± 1.15 ‰), and S. pulchra was most enriched in 15N (2.87 ± 0.95 ‰). Although ecoregion contributed to the variation in δ15N values, those values were not significantly different among ecoregions (F2,40 = 2.65; P > 0.05; Table 1; Fig. 3). Values of δ15N were also not significantly different between early and late season although the total N concentration was significantly greater in early- than late-season forages (F1,320 = 688.11, P > 0.001): graminoids (2.66 ± 0.43 vs 1.32 ± 0.47 gN/100 g DM); woody browse (3.12 ± 0.56 vs 1.34 ± 0.35 gN/100 g DM); Pedicularis spp. (2.91 ± 0.66 vs 1.71 ± 0.82 gN/100 g DM).

The range of values across plant groups was smaller for δ13C than for δ15N (5.60 vs 13.29 ‰, respectively) but variation among species within plant groups was greater for δ13C values than for δ15N values. Plant groups differed in δ13C values (F2,40 = 23.33; P < 0.01; Fig. 2): values for δ13C were highest for graminoids (26.57 ± 1.01 ‰), intermediate in woody browse (27.61 ± 1.25 ‰), and lowest in Pedicularis spp. (28.27 ± 1.02 ‰). Values of δ13C varied among graminoids (Kruskal-Wallis test; P < 0.01), as C. aquatilis was slightly more depleted in 13C (27.39 ± 1.16 ‰) than C. bigelovii

We used analysis of variance (ANOVA) models to examine spatial, temporal, and species-specific changes in values for δ15N and δ13C, with species, ecoregion, and subset (early season or late season) as fixed factors. We tested values of δ15N and δ13C for interactions of species × subset, species × ecoregion, and ecoregion × subset. Values of δ15N and δ13C were log transformed using the inskew procedure in Stata[24] to meet assumptions for normality and tested using the Shapiro-Wilk procedure. The significance of each parameter was assessed with Wald tests.

Linear ordinary least-squares regressions were used to examine the effect of N and C digestibility on isotopic fractionation. Paired t-tests were used to determine the significance of fractionation between diet, indigestible residues, and digested fractions for each forage species.
Effect of nutrient availability on caribou δ¹⁵N and δ¹³C values

Digestibility of N was also greater in early than late season ($F_{1,303} = 115.23; P < 0.001$) for graminoids ($0.59 \pm 0.10$ vs. $0.43 \pm 0.12$), woody browse ($0.42 \pm 0.16$ vs $0.27 \pm 0.13$) and Pedicularis spp. ($0.80 \pm 0.07$ to $0.68 \pm 0.09$). Across plant groups, the fractionation of δ¹⁵N values between the whole plant and the indigestible fraction was negatively correlated with N digestibility ($F_{1,177} = 10.59; P < 0.01$; $R^2 = 0.15$; Fig. 4), with no fractionation when the N digestibility was 0.67. As the N digestibility declined, the residues became significantly more enriched in ¹⁵N than the whole plant especially among species of woody browse (B. nana: δ¹⁵N 2.12 ± 1.21 ‰ and S. richardsonii: δ¹⁵N 0.75 ± 0.44 ‰; both $P \leq 0.01$). This increase in diet-indigestible fractionation with decreasing N digestibility may have been caused by interference from phenolic compounds, because fractionation of δ¹³C values between the diet and the indigestible fraction was correlated with the concentration of phenolic compounds in B. nana ($Y = 0.13x - 0.04; R^2 = 0.62; P < 0.01$) and S. pulchra ($Y = 0.06x - 0.16; R^2 = 0.55; P < 0.02$). In comparison with the diet, digested fractions (available for assimilation into animal tissues) were depleted in ¹⁵N for B. nana (δ¹⁵N −10.48 ± 12.98 ‰; $P = 0.03$), E. vaginatum (δ¹⁵N −0.73 ± 1.31 ‰; $P = 0.04$), and S. richardsonii (δ¹⁵N −0.82 ± 0.56 ‰; $P = 0.03$; Fig. 2).

The digestibility of C was greater in early than late season ($F_{1,199} = 52.50; P < 0.001$) for graminoids (0.34 ± 0.06 to 0.24 ± 0.10), woody browse (0.48 ± 0.05 to 0.40 ± 0.12) and Pedicularis spp. (0.71 ± 0.08 to 0.68 ± 0.11). Fractionation of the δ¹³C values between the whole plant and the digestible and indigestible fractions was also related to forage plant quality. Across plant groups, fractionation of δ¹³C values between the diet and the indigestible fraction was negatively correlated with C digestibility ($F_{1,75} = 40.14; R^2 = 0.30; P < 0.01$; Fig. 4), with no fractionation of the δ¹³C values at a C digestibility of 0.30. As C digestibility increased, residues were more depleted in ¹³C than the whole plant...
(B. nana: δ¹³C = 0.42 ± 0.28 ‰; Pedicularis spp.: δ¹³C = 1.59 ± 0.46 ‰; both P < 0.01). Consequently, the fraction available for assimilation into the animal was more enriched in ¹³C for B. nana (δ¹³C 0.62 ± 0.38 ‰; \( t_9 = -5.14; P < 0.01 \)) and Pedicularis spp. (δ¹³C 0.78 ± 0.35 ‰; \( t_{13} = -8.21; P < 0.01 \); Fig. 2).

**DISCUSSION**

Although both C and N isotopes can be used to discriminate between monocot (graminoids) and dicot (woody browse and Pedicularis spp.) plants, several factors make δ¹⁵N values a more reliable indicator of diet than δ¹³C values for arctic herbivores. The δ¹⁵N values had a 42% greater range than the δ¹³C values for monocot and dicot forage plants, which was consistent with other studies of arctic and alpine plants.⁴,²⁵–²⁷ The values of δ¹⁵N were less affected by temporal variation than those of δ¹³C, which declined over
the season from the Brooks Range to the Coastal Plain, probably due to differing levels of water stress[28–31] and associated effects of seasonal plant growth on stomatal exchanges of gases.[32] Variation in values for $\delta^{15}N$ among species of deciduous shrub probably reflected differences in mycorrhizal associations.[33]

Although differences in growing conditions can create useful distinctions between forage groups, it is still important to consider how those signatures may change during digestion because digestible and indigestible fractions can display differing stable isotope ratios that would then be incorporated into animal tissues and feces. In particular, we found that fractionation varied according to the nutrient digestibility of forage plants. The digestibilities of both C and N showed a negative relationship with fractionation, but significant fractionation between diet and indigestible residues was only observed when the digestibilities were less than 0.67 for N and greater than 0.30 for C. Forage plants of differing digestibility may also be represented in animal tissues at different rates and in different proportions, even if they are consumed at the same rate. For example, Codron et al.[9] found that the incorporation rates of $^{13}C$ were fastest when animals consumed highly digestible diets, and that isotopic compositions of various herbivore tissues were skewed according to the digestibility of the diet. Forbs such as Pedicularis spp. are avidly sought by caribou probably because their digestibility of C and N is high (Fig. 4). The C available for absorption from this forb would be enriched in $^{13}C$ compared with the whole plant and thus its $\delta^{13}C$ values would appear more similar to those obtained from digestion of graminoids. Fractionation of highly digestible forbs may therefore enhance estimates of the contribution of graminoids to the diet when considering the $\delta^{13}C$ values of tissues such as hair.[4] Fractionation of $^{15}N$ during digestion is more likely to affect estimates of diet from indigestible residues especially when the digestibility of N is low (Fig. 3). Estimates of the diet from fecal $\delta^{15}N$ values may be skewed towards graminoids by enrichment of $^{15}N$ in indigestible residues of woody browse species (Fig. 2). These differences in fractionation among plant groups have the potential to bias estimates of consumption when using isotopic analyses of tissues and feces to reconstruct diets over large scales of space or time.[34,35]

Isotopic fractionation is affected by nutrient digestibility through a variety of mechanisms. Waxes on the surface of leaves and lignin in the plant cell wall matrix are resistant to digestion by acid and enzymes.[36] The lower $\delta^{13}C$ values in indigestible residues of forages are consistent with the $\delta^{13}C$ values of lipids and lignin that are typically lower than those of whole leaves.[32] Digestion of N is affected by physical access of enzymes to substrates, inhibition of the enzyme, and the affinity of the enzyme for the substrate, all of which can influence N fractionation.[36] Enzyme affinity for substrate proteins probably has little effect on fractionation because protease activities are high for a wide variety of dietary proteins and because most of the plant protein is present in the form of a single photosynthetic protein, Rubisco.[37] Physical access and enzyme inhibition probably account for most of the fractionation due to the actions of PSMs such as tannins that can both limit physical access of dietary enzymes to protein and inhibit the dietary enzymes themselves[38,39] depending on the binding affinity between tannins and proteins in the digestive tract.[40] Fractionation of N in woody browse may therefore depend upon the suite of PSMs, which changes according to species, ecotype, and season. Enrichment of $^{15}N$ in indigestible residues of woody browse would enhance the $\delta^{15}N$ values of fecal residues and the effect of microbial colonization of fibrous residues on fecal $\delta^{15}N$ values.[31]
CONCLUSIONS

Fractionation between diet and indigestible residues reduces isotopic separation between browse and graminoids (Fig. 2) and thus decreases the estimated contribution of heavily defended shrubs such as _B. nana_ to the diet of arctic caribou and other arctic ungulates. Mixing models that estimate diet from the isotopic ratios of herbivore tissues would be improved by including the range of fractionation values when the diet includes highly digestible items (e.g. forbs) or forages rich in PSM (e.g. woody browse).

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